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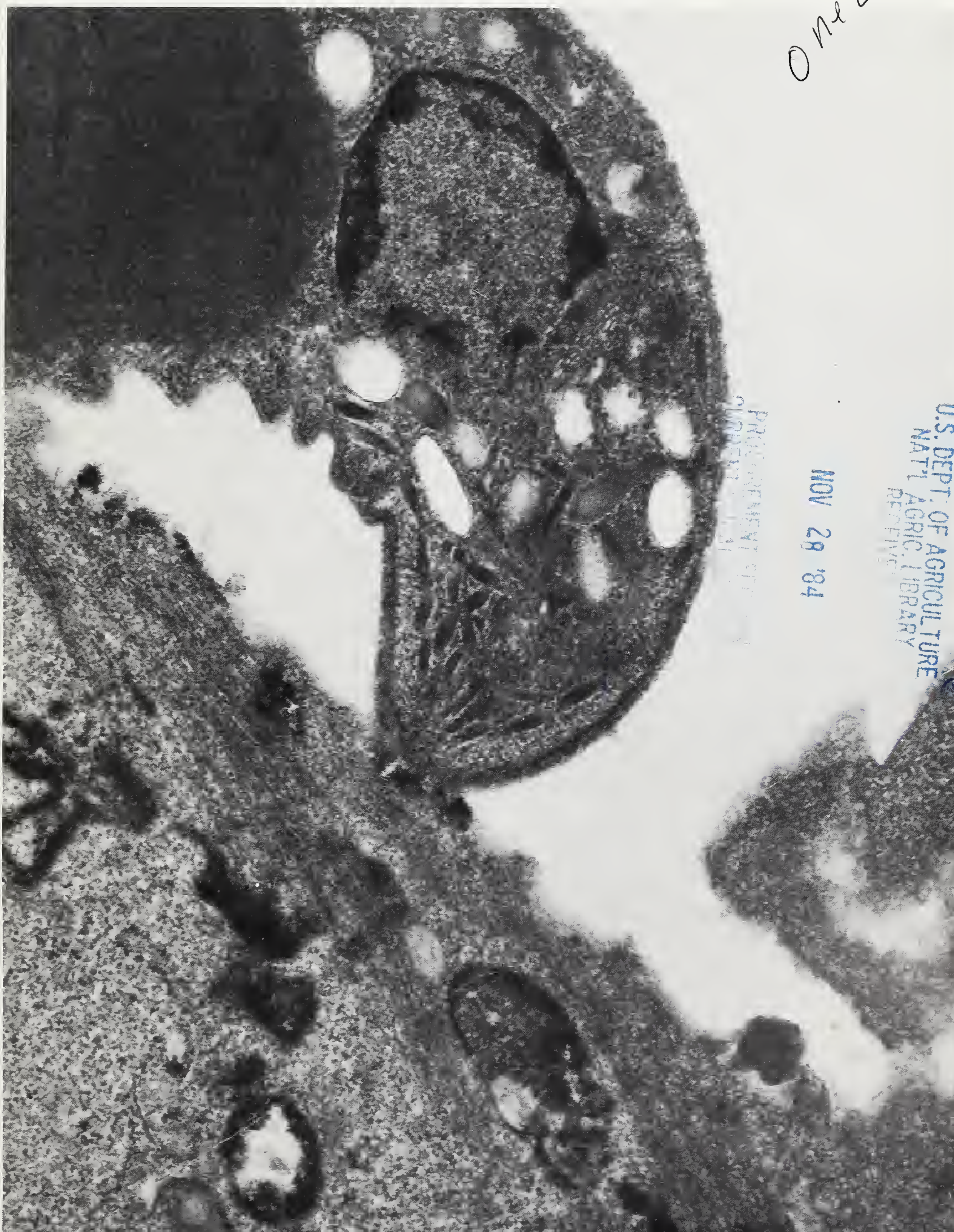
Agricultural Research



Demystifying Membranes: The Ninth Beltsville Symposium

For 4 days this past May, the rule of caution on scientific speculation was relaxed a bit as more than 300 of the world's leading scientists in research on biological membranes met to share insights, and even some big-scale dreaming, at the Ninth Beltsville Symposium.

Scientists have studied biomembranes—the filmlike inner sacs of living cells and cell parts—for 100 years. In very recent years, however, an array of sophisticated laboratory tools has suddenly opened new paths of molecular exploration on the mystical membranes of life.

Using nuclear magnetic resonance, X-ray diffraction, high resolution electron microscopy, and a host of other techniques, scientists are gaining some highly specific knowledge of membrane functions that may be keys to improving food and fiber production. (See this month's feature story, p. 8.)

Plant parts that react and act . . .

Biomembranes are commonly composed of two layers of fatty or lipid materials. They are bathed on each side by fluids that regulate the passage of water-soluble substances into and out of cells or cell parts. Also, embedded in membranes are protein complexes that control highly specific reactions.

Membranes (1) provide semipermeable barriers to limit mixing of liquid environments of cells and intercellular spaces, (2) are sites for chemical reactions, and (3) act as communications channels among cells.

Membrane research may play a large part in helping plants withstand environmental stress. At least 30 percent of potential U.S. crop yields are lost to drought, extreme temperatures, salinity, or other stresses. Researchers have found that of all structural cell parts, membranes are both the most sensitive to the environment and the only parts that can do something about it.

Plant cells have a sort of a rapid transit system to rush first aid in the form of fatty acid rearrangements to external membranes when the weather suddenly turns hot or cold. Research has also revealed a slower, "backup system" whereby plant cells desaturate fatty acids to cope with stress.

In the area of animal parasites, researchers have found that causal agents of such diseases as coccidiosis in livestock and malaria in humans may recognize and exploit specific sites on membranes to invade a victim's cells. Scientists have isolated and purified some site proteins and have cultured and tested antibodies against the membrane proteins. These monoclonal antibody cultures may lead to vaccines or other treatments to inhibit parasite invasions.

"Cracking the case" of how photosynthesis operates in green leaves is particularly exciting to agricultural researchers, many of whom look to photosynthesis enhancement in crops as the most promising angle on making crop yields jump to new plateaus.

The focal point of research is the membrane of the chloroplast, the cellular home of the green pigment chlorophyll. Molecules of the pigment are the light-absorbing antennae of plants. By measuring ratios of different types of molecules, researchers have shown that a series of electron-transfer reactions that convert light energy to chemical energy in plants take place at sites on the chloroplast membrane. Such energy transfer in crop plants has a direct impact on yield.

Another recent discovery is that aluminum molecules can disable a protein linked to the well-being of plant cells. Since perhaps 40 percent of the world's arable soils can potentially cause aluminum toxicity to crops, researchers are pursuing ways to prevent aluminum damage to the protein, perhaps by breeding plants with higher levels of certain organic acids.

Membranes and genetic engineering . . .

In the opinion of the scientists attending the symposium, membrane research will help fuel genetic engineering breakthroughs in 21st-century agriculture.

The fruits of such research may be crops that withstand severe weather, resist herbicides, and are more efficient in converting light energy into sugars and starches during photosynthesis. New knowledge should help nutritionists improve the ability of humans and animals to derive nutrients from their diets. And we will be able to protect animals from parasites, and plants from toxins wielded by certain pathogens.

The individual scientist may be unwilling to cast such wide projections during day-to-day work at the bench. But the symposium was the right time and place to acknowledge that such dreams are now within the realm of possibility.

Stephen Berberich
Beltsville, Md.

Note: *Due to a typographical error, the July-August issue carried the same number as the June issue, Vol. 32, No. 10. July-August is actually No. 11. Also, we wish to inform our readers that Vol. 32 will end with the November-December issue, No. 14. Vol. 33, No. 1, will be the January 1985 issue. The volume year will correspond with the calendar year thenceforth.*

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Cover: An *Eimeria* parasite begins to penetrate a vulnerable point on the membrane of a turkey kidney cell. Burgeoning knowledge of the function and role of membranes—afforded by new, high-tech lab techniques—is beginning to benefit many areas of agricultural biotechnology. Story begins on p. 8. (PN-7131)

GRIN All the Way to the Gene Banks

Any plant scientist with a computer terminal and a telephone has much of the nation's plant resources at his or her fingertips.

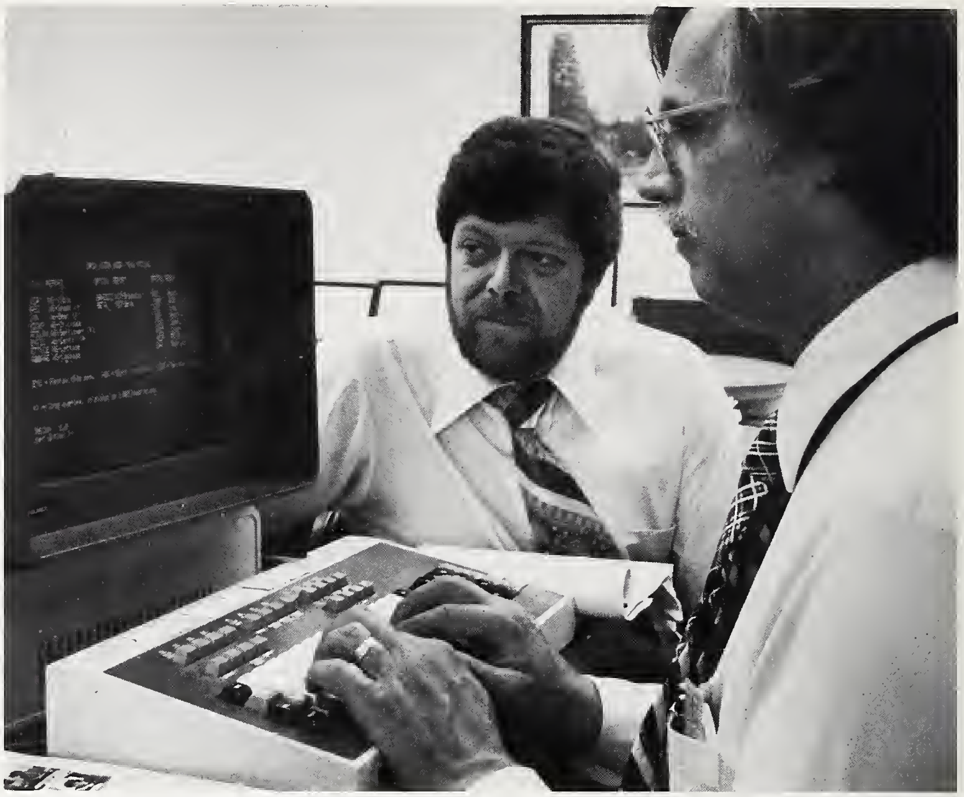
The Germplasm Resources Information Network (GRIN), an electronic seed/plant catalog managed by ARS, is now on line. The product of 7 years of planning, GRIN will serve public and private researchers and the curators of germplasm (plant gene) collections. It contains information on the characteristics of over 600,000 plant samples collected worldwide.

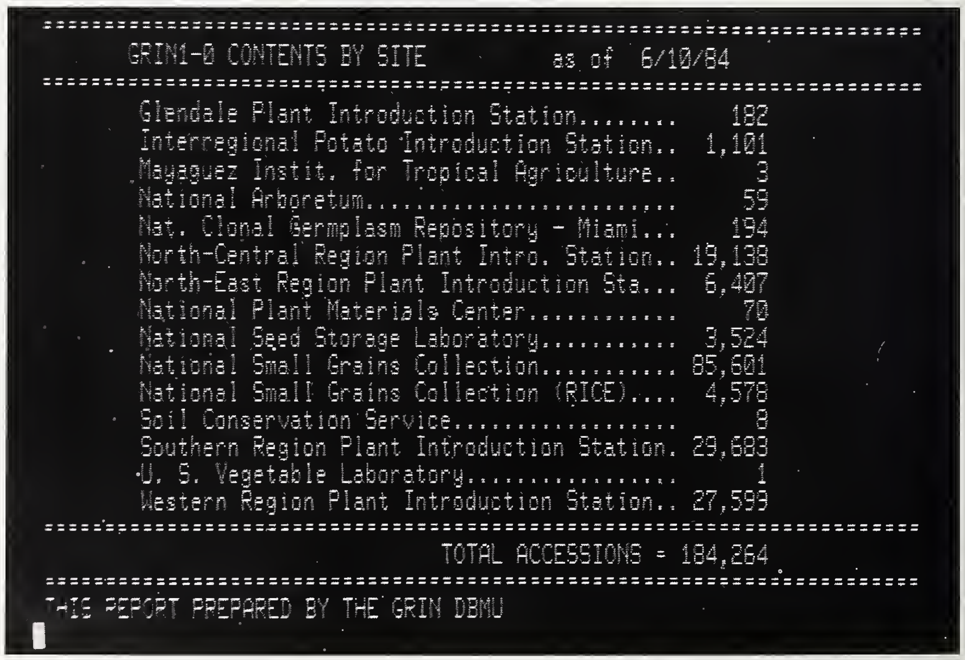
The network—largest of its kind—is a powerful tool for plant scientists, according to Allan Stoner, chairman of the Plant Genetics and Germplasm Institute, Beltsville, Md., the design and program hub of GRIN. It can be easily accessed, says the institute's computer specialist Jimmie Mowder, by any site in North America with a remote terminal, telephone lines, a printer, and a user password available from the GRIN center in Beltsville.

Scientists hooked into GRIN can quickly locate combinations of plant genes to help them develop crops that will better cope with diseases and insect pests, harsh climates, or poor soils, says Stoner. The network merges data of many collections of seeds and live plants of the National Plant Germplasm System.

To breed a better bean, for example, a scientist sifts through GRIN data displayed on a computer screen, extracting traits relevant to his or her plan. In minutes, the network identifies plant heights, nutrient contents, seed color, and 54 other traits for nearly 10,000 types of snap or *Phaseolus* beans stored in Pullman, Wash.

GRIN includes data on traditional or farmers' varieties from around the world that have been replaced by higher yielding varieties but may contain genes useful to breeders. It also contains data on wild and semiwild plants. For instance, scientists recently collected seed from semiwild grasses in the Middle East that proved to be the same types of wheat, rye, and barley crops cited in the oldest chapters of the Bible. The seed, valued by breeders for unusual resistances to some of today's most devastating cereal diseases, is now preserved in ARS's Small Grains Collection at Beltsville.





GRIN1-0 CONTENTS BY SITE as of 6/10/84

Glendale Plant Introduction Station........	182
Interregional Potato Introduction Station..	1,101
Mayaguez Instit. for Tropical Agriculture..	3
National Arboretum.......................	59
Nat. Clonal Germplasm Repository - Miami...	194
North-Central Region Plant Intro. Station..	19,138
North-East Region Plant Introduction Sta...	6,407
National Plant Materials Center...........	70
National Seed Storage Laboratory..........	3,524
National Small Grains Collection..........	85,601
National Small Grains Collection (RICE)....	4,578
Soil Conservation Service.................	8
Southern Region Plant Introduction Station.	29,683
U. S. Vegetable Laboratory................	1
Western Region Plant Introduction Station..	27,599

TOTAL ACCESSIONS = 184,264

THIS REPORT PREPARED BY THE GRIN DBMU

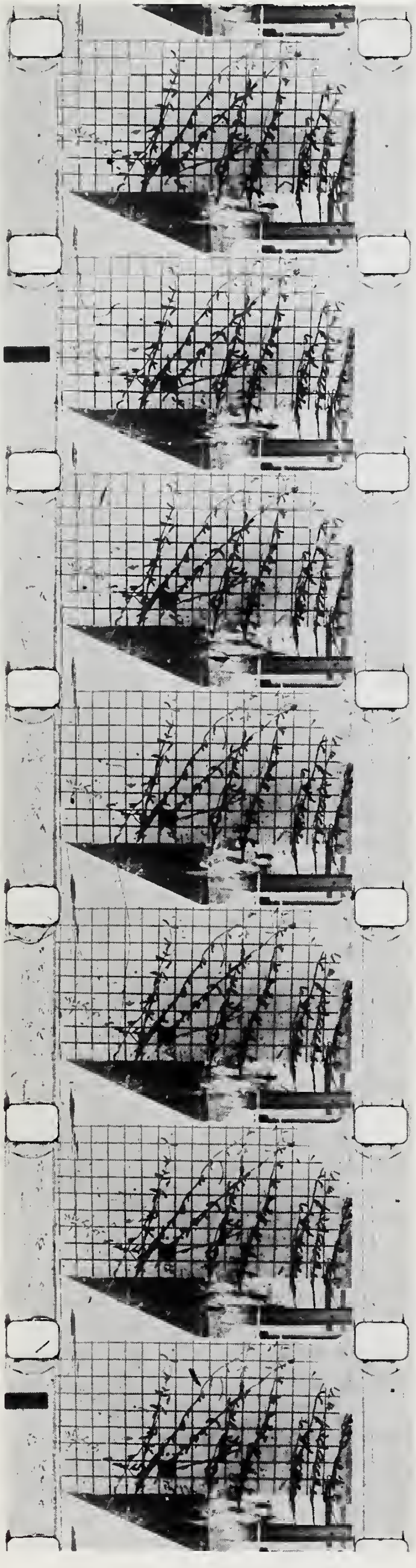
"Put simply, GRIN helps ensure a diversity of germplasm for scientists to keep cultivated crops dynamic and productive," says Stoner.

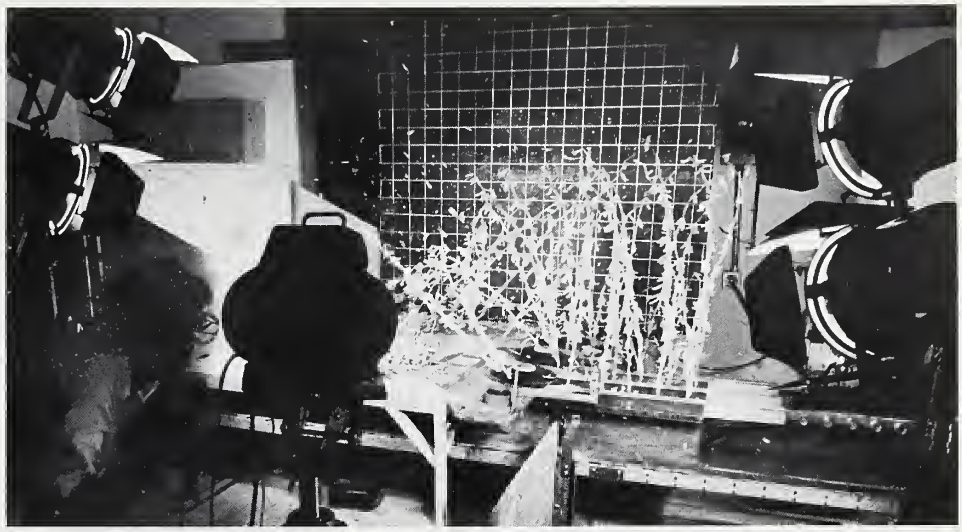
Allan Stoner is located in Rm. 127, Bldg. 001, Beltsville Agricultural Research Center—West, Beltsville, Md. 20705.—**Stephen Berberich,** Beltsville, Md. ■

Top: At the Plant Genetics and Germplasm Institute in Beltsville, Md., project administrator John Belt (left) instructs Maryland University botanist James Reveal how to access the Germplasm Resources Information Network. (0684W926-27A)

Above: A computer terminal displays the number of plant germplasm samples residing in the GRIN database. (0684W924-8)

Rotary Disk Mower Reduces Soybean Harvest Losses





Top: By using a super-high-speed motion picture camera to capture the action, agricultural engineer John W. Hummel can actually see a harvester blade cut into a soybean stem. The film will help Hummel investigate how the impact of the blade against the stem causes some of the pods to open and drop their beans. (0884X1126-4) Frames from one of the films are shown at left. (PN-7133)

The next major advance in soybean harvesting may allow farmers to bring in about 2 percent more of their crop than they now can with today's combines with row-crop headers.

According to agricultural engineer John W. Hummel, the annual savings would total an estimated $246 million, based on last year's soybean harvest.

That extra income could come from impact-cutting beans with a rotary disk mower. This technique cuts and ''airlifts'' soybeans into the harvesting machine so fewer land on the ground, says Hummel, who is located in Urbana, Ill.

V-shaped teeth on sickle bars, used today for soybean harvesting, move rapidly back and forth to cut stalks but jar loose too many beans, which the harvester then misses, he says.

Hummel has been taking high-speed movies—as fast as 5,000 frames a second—to document what happens in the new cutting technique.

As the whirling disks and knives hinged to the edges cut off the stalks, the curved disks help float the beans that break free. That airlift keeps a larger percentage of beans aloft long enough to be harvested instead of lost.

Use of the rotary disks may someday save half the soybeans that farmers now lose while combining. About 4 percent of last year's 1.6 billion bushel soybean crop was left on the ground, he says.

Hummel has captured on film the cutting and airlifting of beans hundreds of times in laboratory experiments of the rotary disk at the University of Illinois. He freezes the disks, spinning at 170 miles an hour, in split-second images to see how they cut plant stalks.

One of the problems in cutting soybean plants, he says, is that they are physically unlike small grain crops typically harvested by combines. Their large pods, which grow along the entire stalk, shatter and lose beans when jarred or shaken.

Hummel uses shutter speeds of 500 frames a second to track the trajectory of plants entering the harvester. He speeds the filming to 5,000 frames a second to capture loose beans in the air and to freeze the movement or acceleration of plant stalks as they are cut.

His harvester mock-up has three rotary disks, each measuring 13.5 inches in diameter, spinning horizontal to and about 2 inches above the ground.

In the laboratory tests, soybeans as dry as those normally harvested were mounted in rows on a carriage bed, then driven between the cutting disks at three simulated travel speeds for the field. Harvest losses were lowest at the highest speed, 8.9 miles an hour, Hummel says.

John W. Hummel is located in Rm. 105, Agricultural Engineering Bldg., 1028 W. Peabody, University of Illinois, Urbana, Ill. 61801.—**Ben Hardin,** Peoria, Ill., and **Russell Kaniuka,** Beltsville, Md. ■

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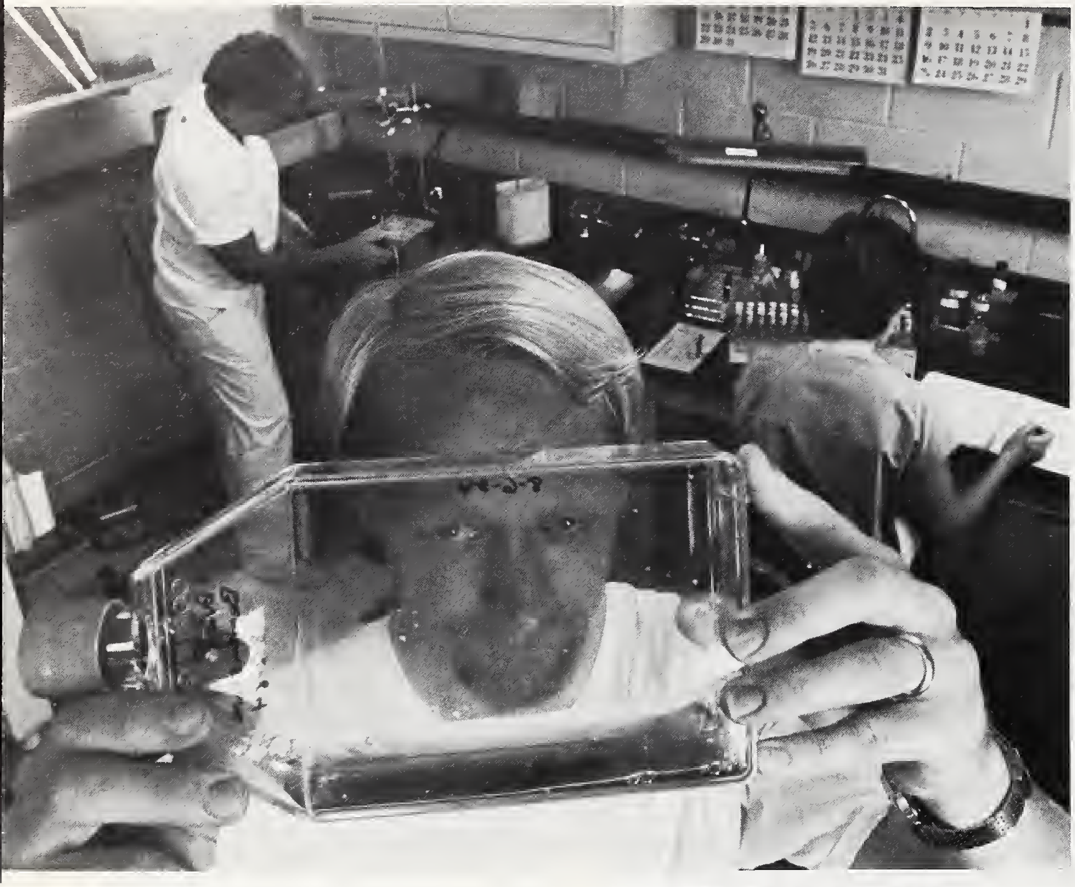
Viral One-Two Causes Fatal Cattle Disease



Above: Veterinary scientist Steven Bolin checks a bottled culture of bovine viral diarrhea (BVD) virus while lab technicians Sharon Stark (left) and Shari Steadham prepare additional blood samples from infected cattle. (0884X1120-25)

Right: On the display screen of cell counting and measuring equipment, Bolin and lab technician Vickie Hall compare profiles of white blood cell types from animals persistently affected with BVD virus. (0884X1122-12)

Opposite page: The calves Bolin is studying may appear healthy, but their mother (at left) was exposed to BVD virus early in pregnancy. Only with blood tests of her offspring can Bolin determine whether any of them carry the fatal virus. (0884X1121-28)

The one-two punch may be famous in the world of boxing, and a virus uses it with deadly effect in the world of cattle.

The viral one-two causes a fatal cattle disease that was first described in 1946. Only now has its lethal action been duplicated in the laboratory, according to veterinary scientist Steven R. Bolin at the National Animal Disease Center.

Experiments confirm that a first attack—a persistent infection hidden in apparently healthy animals—makes animals susceptible to a second attack from the same virus, causing fatal bovine viral diarrhea or mucosal disease.

The first attack, before the fetus is born, knocks down the defense. It may cause the cow to abort or cause the calf to die shortly after birth. In surviving calves the virus persists for life, even invading the animal's white blood cells, Bolin says. These survivors carry the virus but no antibodies to it in their blood serum. They appear healthy and live without defense until they are exposed to the virus a second time, either naturally or through vaccination. The second attack kills with mucosal disease, a name derived from the damage to the mucous membrane and mucus encrustation on the nose.

Breeders unaware of the hidden persistent infection in their cows may keep their apparently healthy calves as breeding stock, says Bolin, thus building vulnerable herds over time.

Identifying infected animals with blood tests and culling them would remove cattle susceptible to the second attack and "might prevent losses due to severe bovine viral diarrhea," Bolin says. However, "it is difficult to assess the economic importance of our findings," he says. "We don't know the prevalence of the persistent infection because it is hidden in apparently healthy cattle."

Retired veterinary scientist Arlan W. McClurkin and microbiologist Manuel F. Coria discovered the hidden infection more than 10 years ago. From an apparently healthy bull calf that had no antibodies to the disease they isolated a noncytopathic virus—a strain of the virus that does not kill cells. The cytopathic—cell-killing—strain of the same virus causes mucosal disease: part two of the one-two punch.

In the latest development from 10

years of research, Bolin, McClurkin, Coria, and veterinary scientist Randall C. Cutlip consistently produce mucosal disease with the viral one-two. They infect the fetus before it is 125 days old with the noncytopathic virus to cause the persistent, hidden infection. Then they infect the apparently healthy survivors with the cytopathic strain to cause mucosal disease. If exposed to the virus after 125 days, the fetus can produce antibodies that protect it against both strains, Bolin says.

Veterinary scientists E. Travis Littledike and Glynn H. Frank joined the team in producing the persistent, hidden infection. They exposed 42-to-125-day-old fetuses to both virus strains. McClurkin says the studies show that the noncytopathic strain persists and causes the hidden infection. The cytopathic strain does not.

The studies also show that either inoculating fetuses in cows carrying antibodies or inoculating pregnant cows not carrying antibodies may produce infected calves that appear healthy. McClurkin calls such survivors "a constant source of infection for other cattle."

He says "the ability to produce persistently infected animals by exposing fetuses at various stages of gestation shows the need for an inactivated vaccine to control the disease."

In their earlier research, McClurkin, Coria, and Cutlip studied the effects of hidden infection on reproductive performance. When cows with antibodies to the virus were bred to a bull carrying the noncytopathic virus, they had normal calves.

When heifers without antibodies were bred to the bull, they developed antibodies within 2 weeks and had high serum antibody concentrations by 6 weeks. One heifer conceived after one service but aborted. Four others had calves that appeared healthy but were without antibodies to the virus.

In addition to producing the hidden infection and mucosal disease experimentally, the team isolated both noncytopathic and cytopathic virus from spleens of cattle killed by bovine viral diarrhea. Isolating both strains from field cases supports the one-two explanation, McClurkin says. Preliminary reports of similar research in Great Britain support it also.

Presence of antibodies to bovine viral diarrhea virus shows that it infects 65 to 85 percent of cattle all over the world, McClurkin says.

The usual form of the persistent infection is mild. Animals have slight diarrhea, may lose appetite, run elevated temperatures 12 to 48 hours, and recover when they develop antibodies. If infected after 125 days, fetuses develop antibodies and are born with immunity.

The infection takes at least three other forms, McClurkin says. Infection in the first 125 days may cause abortion, short life after birth, or the hidden, persistent infection that leaves survivors without antibodies to the virus and vulnerable to the other two forms of the disease—acute or chronic fatal infection.

In both forms, animals run a fever, breathe rapidly, and have fewer than the normal number of white blood cells. They lose appetite and have bloody diarrhea. If the disease is acute, they die within 5 to 7 days.

If it is chronic, they live 10 days to a few months, long enough to develop symptoms that give the name to mucosal disease. Bolin says the mucous membranes erode and ulcerate and mucus encrusts the nose.

Steven R. Bolin is located at the National Animal Disease Center, P.O. Box 70, Ames, Iowa 50010.—**Dean Mayberry**, Peoria, Ill. ■

Membrane Research— A Multidisciplinary Treasure Trove

Plant physiologist Gregory Taylor, with ARS on a fellowship from the Canadian Natural Sciences and Engineering Research Council, examines the roots of a wheat variety particularly sensitive to aluminum toxicity. (0984X1353-25A)

Biology, one of the parent disciplines of science, is also the foundation of many of today's industries and businesses. The fields of medicine, nutrition, and agriculture are all built upon knowledge of biology, and the research pioneers in each area are becoming more and more specialized.

However, scientific interest in biological membranes cuts across specialty lines. Biomembranes—the "gatekeepers" of life processes in cells—are fluid, film-like surfaces, one to two molecules thick, that control the chemistry and physics in and around cells.

Current research on biomembranes, according to plant physiologist Meryl Christiansen, often has a refocusing effect on today's diverse life scientists. This past May, Christiansen served as chairman of the 1984 Beltsville Agricultural Symposium, "Frontiers in Membrane Research in Agriculture."

The symposium showcased a revolution in biotechnology, says Christiansen, in which new, high-powered laboratory instruments are giving scientists a much clearer look at the essential work performed by membranes in virtually all life forms.

Presentations to over 300 of the world's leading membrane scientists in attendance sparked cross-disciplinary discussions. A nutritionist pinpointing places on membranes where vitamins probably enter human intestinal cells held the attention of animal scientists. A plant pathologist describing how a fungus may wield toxins as weapons to punch through plant cell membranes piqued the interest of microbiologists. And so it went.

Three of the ARS projects presented at the symposium illustrate the scope of membrane research on major agricultural problems. The projects address (1) soil-caused metal toxicity of crops, (2) crop-breeding limitations in the face of mounting world food needs, and (3) livestock losses from deadly parasites. Early findings in each area hold promise for new solutions, thanks entirely to the new and ever-growing knowledge of biomembranes.

Secrets of Aluminum Poisoning of Crops

Detectives in biochemistry may soon close a case that has puzzled agricultural researchers for at least 66 years—crop poisoning by soil aluminum.

Plant physiologist Charles R. Caldwell and co-workers at the Plant Stress Laboratory, Beltsville, Md., have unearthed new clues as to how molecules of aluminum break and enter cell membranes in plant roots. Knowing the molecular reasons why crops are stunted by aluminum could speed up breeding of aluminum-tolerant crops, says Caldwell.

Plant poisoning by aluminum, the most abundant metal in the earth's crust, is a global problem for farmers. In highly acidic soils, aluminum becomes active, moving into plant roots. Crops on the roughly 40 percent of all arable lands (or up to 70 percent of potentially arable lands) that are acidic can be stunted by aluminum toxicity.

Sophisticated analytical equipment helped Michigan State University biophysicist Alfred Haug and Caldwell show that aluminum profoundly disrupts cell membrane integrity. Aluminum compounds appear to irritate specific locations or sites on membranes, which react by allowing the compounds to enter the cells.

Experiments have shown that once inside cells, aluminum binds to a protein called calmodulin, an acronym for *cal*cium *modul*ating prote*in*. In all organisms except some bacteria, calmodulin regulates the cell's life-or-

death functions, many linked to the essential mineral calcium.

When bound to aluminum, calmodulin tends to stop regulating energy that allows the growth pigment, phytochrome, to work properly; that helps maintain cell walls; that genetically prepares cells for division; and many other important cell functions, say the scientists.

But not always. In the tea plant (*Camellia sinensis*), for example, an abundance of organic acids, notably citric acid, holds aluminum away from calmodulin, thus protecting cells from the brunt of aluminum toxicity.

The organic-acid effect, the scientists say, may provide a rapid screening method for locating genes that control aluminum tolerance. Large amounts of certain organic acids in some plants could be a tipoff to researchers to test for tolerance.

In other work at the Plant Stress Laboratory, comprehensive research is aimed at helping agriculture tailor plants to aluminum-containing soils. Soil scientist Charles Foy has found dramatic differences in aluminum tolerances among crop species, varieties, and even among strains or individual plants of one variety.

"Now that we can measure precisely what happens at the membrane level, the tremendous variances in aluminum tolerances in plant germplasm can soon be exploited by breeders," says Foy.

Parallel research underway worldwide in genetic and cell culture engineering may in turn lead to ways to select aluminum-tolerant breeding stock from test-tube nurseries. Scientists break down plant tissue into individual cells and keep the cells alive in dishes of nutrient solution. Each cell can possess unique sets of genetic traits not yet expressed in a complete plant.

Cell culture techniques allow scientists to select cells for desired traits, then regenerate whole plants from selected cells. In order to produce an aluminum-tolerant plant from cell culture, says Caldwell, researchers will flood cultures with increasing amounts of aluminum compounds until only a few cells are still alive. Those few could be tomorrow's breeding stock for aluminum-tolerant varieties.

Finding genes for aluminum tolerance, high yield, disease resistance, or other good traits is, of course, only preliminary to breeding for crop improvements.

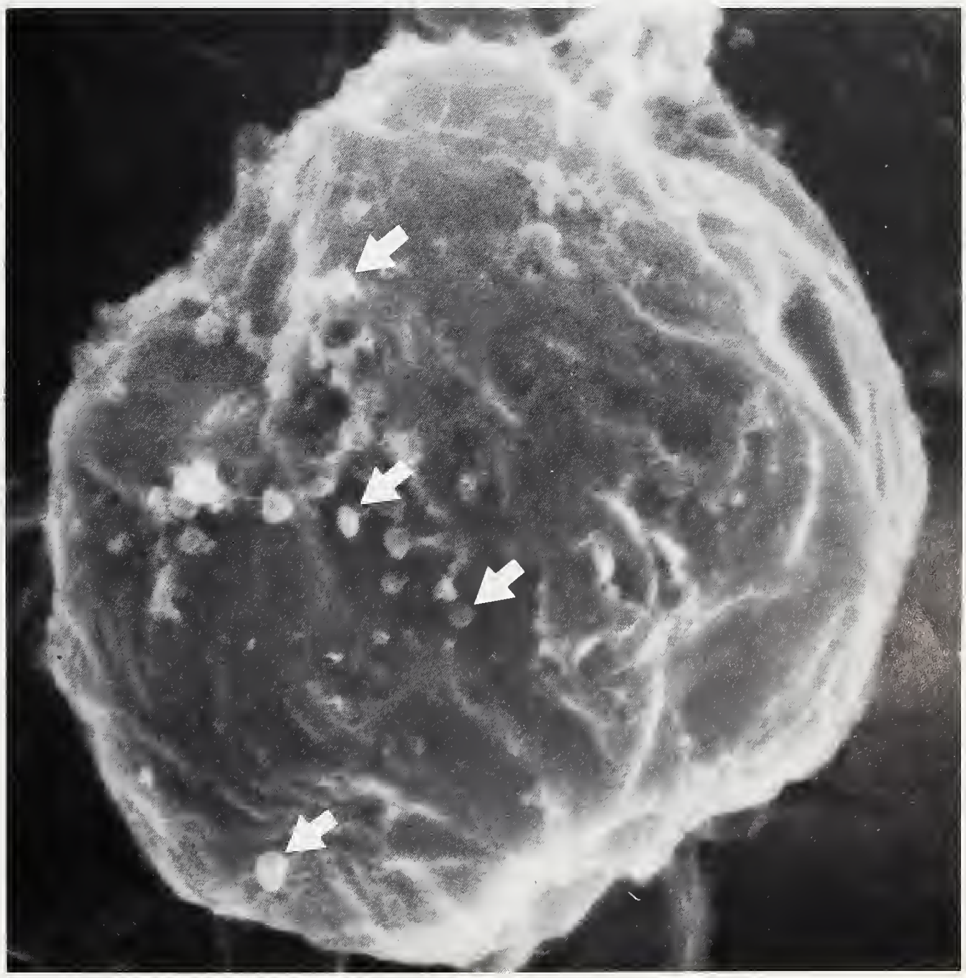
Liposomes—Artificial Membranes

Liposomes (marked by arrows) are hollow spheres made of fat molecules. They closely resemble cell membranes and have been used for more than two decades as models to study the structure and general properties of membranes.

Because liposomes are hollow, they can encapsulate compounds. Because they can fuse with plant and animal cells under certain circumstances, researchers use liposomes as vehicles for carrying genetic material into cells. In this photograph, the tiny liposomes are carrying plant DNA that scientists wish to insert into a carrot cell (the much larger sphere). The carrot cell has had its walls removed.

Liposomes can also be used to encapsulate antibiotics and other drugs for long-term treatment of diseases in farm animals.

Research advances are creating a new generation of liposomes than can be better tailored to specific tasks. Liposomes' amazing array of properties will make them increasingly important investigative tools in disease diagnostics, genetic engineering, and animal health care.



(PN-7130)

Plant physiologist Charles R. Caldwell sets up specialized equipment that uses lasers to measure changes in plant cell membranes subjected to aluminum toxicity. (0884W1308-18A)

Cell fusion experiments by biochemist James Saunders and student assistant Kinlin Choa could lead to plants with new genetic combinations. (0884W1306-9A)

However, techniques under development for transferring genes between cells of two plants that cannot cross in nature could open new worlds to crop breeders.

Shocking Membranes for Genetic Engineering

Using chemicals to fuse cells from different plants is one way scientists have already been combining genes; however, this technique has had only a 2- to 3-percent success rate.

Recently developed electroshock fusion of cells can produce 100 percent viable cells for experiments.

In a Beltsville laboratory, biochemist James A. Saunders has been lining up plant cells—stripped down to "bare" membranes—in an electrofusion chamber, a 4-inch platform mounted under a microscope. Saunders adjusts tiny points of AC (alternating current) electrodes until the cells' membranes are snug to one another. Then he throws a switch. Zap! A rush of DC (direct current) jolts the membranes, causing them to fuse. Pairs of plant cells become single, larger cells. The genes of cell pairs mix considerably in fused cells.

Saunders' research is contributing to the development of "blueprints" of membrane compatibilities for fusing cells. His experiments on cross-species fusing could lead to an explosion of new genetic trait combinations.

Although the transfer of genes by fusion is not precise, "we are taking on the challenge of learning how to control and use desirable traits, such as aluminum tolerance, and to toss out unwanted ones," he says.

Fusing cells is also a useful technique in research on animal tissues, specifically of diseases and parasites and their intimate links with membranes.

Closing Cell Doors to Parasites

Some very small parasites can slip harmlessly past membranes and into living cells of animal and human intestines, where they multiply.

Then, when exiting, the parasites burst back through the membranes, destroying the cells. "They are not such nice guys on the way out," says microbiologist Patricia Augustine, who is working on try-

ing to block parasite entrance to cells.

Augustine and fellow microbiologist Harry Danforth, both at the Protozoan Diseases Laboratory, Beltsville, Md., are investigating the one-celled parasite *Eimeria,* the causal agent of chicken coccidiosis. Each year the disease costs U.S. poultry producers more than $150 million in diseased birds and about $10 million in anticoccidial drugs.

Augustine and Danforth are using electron microscopy and monoclonal antibody techniques (see *Agricultural Research,* Jan. 1984, p. 8) to learn how micro-invaders such as *Eimeria* "jimmy open" doors in cell membranes. The research is aimed at finding alternatives to expensive and not always reliable vaccines and drugs. Such treatments, although preventive, become obsolete when parasites adapt genetically to resist the chemicals.

Blocking cell entry by *Eimeria* depends on pinpointing membrane receptor sites visited by the parasite, says Augustine. In nature, *Eimeria* would soon die without access to specific turkey or chicken cells, which helps explain why experiments indicate that the parasite plays an active role in cell penetration.

As part of their approach to finding the parasite's entry points on membranes, Augustine and Danforth developed monoclonal antibodies (MA) against chicken cells. Two of nine MA preparations tested inhibited parasite invasions of chicken cells in laboratory culture by 36 to 50 percent. The scientists are continuing to use monoclonal antibodies to help them zero in on sites where the parasite can slip through.

Thanks to pioneering research in biomembranes, small gut parasites such as *Eimeria* may one day find their favorite doors into cells blocked by patrolling vaccines.

Meryl Christiansen is located in Rm. 221, Bldg. 001; Charles R. Caldwell in Rm. 15, Bldg. 001; Charles Foy in Rm. 211, Bldg. 001; James A. Saunders in Rm. 116, Bldg. 001, Beltsville Agricultural Research Center—West, Beltsville, Md. 20705. Patricia Augustine and Harry Danforth are located in Bldg. 1040, Beltsville Agricultural Research Center—East, Beltsville, Md. 20705.—**Stephen Berberich, Annelle Black,** and **Vincent Mazzola,** Beltsville, Md.■

Microbiologists Patricia Augustine and Harry Danforth compare electron micrographs of cell membrane-parasite interactions. (0884W1303-16A)

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Giving Peaches a New Lease on Shelf Life

Plant pathologist Lawrence Pusey coated the peaches on the right with a suspension of *Bacillus subtilis* that protected them against brown rot disease, which ravaged those on the left. (0684W845-19A)

Thrown away any fresh peaches lately because a fuzzy blackish fungus got to them before you could? You're not alone.

Brown rot is the most pernicious disease of peaches, apricots, and nectarines worldwide. Growers or packers must apply a fungicide after harvest to keep the fruits in fresh-market condition. But the chemical loses its punch in less than 2 weeks, leaving grocers or consumers to cope with the disease, which begins as small brown spots on the fruit.

Now, in some unique research, an uncommon strain of a common bacterium is rivaling fungicides in keeping the fungus at bay. The bacterium, a variant of the naturally occurring *Bacillus subtilis,* produces a very potent antibiotic that is nearly as effective against brown rot fungi as the widely used fungicide benomyl.

According to its discoverers, plant pathologists Charles L. Wilson and P. Lawrence Pusey, the bacterium could be applied to stone fruits at the market and thereby extend the shelf life up to 2 weeks. The bacterium and its antibiotic have yet to undergo safety testing, but the scientists expect them to be declared safe for human consumption, pointing out that several variants of another bacterium, *Bacillus thuringiensis* (commonly known as B.t.), have been registered for use on produce right up to harvest.

During 2 years of tests at the Appalachian Fruit Research Station, Kearneysville, W.Va., the bacterium performed well at room temperature on all stone fruits—peaches, apricots, nectarines, plums, and cherries—and within a wide temperature range (5°–30° C or 41°–86° F) on peaches.

What's more, the bacterium produces very stable spores that can withstand environmental extremes. This will make it easy for a manufacturer to package the biological control agent and for a grocer to apply it, explains Pusey, who is continuing this research at the Southeastern Fruit and Tree Nut Research Laboratory in Byron, Ga. The organism also performs well when combined with a mineral oil, paraffin-based wax that packers apply

to peaches just before shipping to prevent dehydration. According to Pusey, about half of the packers in Georgia now combine fungicides with the wax treatment.

Under orchard conditions, the bacterium would probably not maintain a population high enough to control brown rot, says Pusey, but "we've demonstrated the potential for using biologicals in storage or in the market where the environment can be controlled."

The ARS researchers became involved in this work to find alternatives to chemical fungicides—currently the only effective deterrent of brown rot and other postharvest diseases. Wilson contends that the chemicals pose a potential health hazard. Moreover, they are becoming less effective as resistant strains of the brown rot fungi become more widespread.

Wilson and Pusey are gearing up for a 3-year project in which they plan to develop reliable techniques for commercial-scale use of biological control agents. Because the bacterium performs better when combined with its favorite nutrients, they will look for ways to incorporate nutrients and antibiotic stabilizers into the treatment process. At the same time, they will search for organisms that can top *B. subtilis* in performance—a definite possibility, according to Pusey.

Although the technology is still on the drawing board and safety testing needs to be done, the scientists have applied for a patent on their discovery, which means that commercial businesses may obtain a license on the process. It is Patent Application Serial No. 606,069, "Postharvest Biological Control of Stone Fruit Brown Rot by *Bacillus subtilis.*" For technical information, contact the scientists at the addresses listed below. For information on obtaining a license on the patent, write to: Patents Office, USDA-ARS, Office of the Administrator, Rm. 323, Bldg. 003, Beltsville Agricultural Research Center—West, Beltsville, Md. 20705.

Charles L. Wilson is located at the Appalachian Fruit Research Station, Rt. 2, Box 45, Kearneysville, W.Va. 25430. P. Lawrence Pusey is located at the Southeastern Fruit and Tree Nut Research Laboratory, P.O. Box 87, Byron, Ga. 31008.—**Judy McBride,** Beltsville, Md. ■

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Breast-Fed Preemies Get a Bonus

Mothers of premature babies produce milk that is easier to digest and more suitable to developing the newborn's brain and nervous system than milk of mothers who deliver full-term infants.

These findings, says chemist Joel Bitman, are important in understanding the nutritional requirements of the premature infant and for the proper handling of milk in human milk banks.

In a joint study with Margit Hamosh of the Georgetown University Medical School's Department of Pediatrics, Washington, D.C., Bitman investigated the composition of breast milk from mothers of 18 very premature, 28 premature, and 6 full-term infants.

Bitman, assisted by chemist Larry Wood, found that milk from mothers delivering babies 1 to 3 months early contained nearly twice as many long-chain polyunsaturated fatty acids. These polyunsaturates are believed to be vital in the growth of brain cells and the formation of lining around nerve fibers, explains Bitman.

The study also showed that milk from these mothers had about 70 percent more of the easily assimilated medium-chain fatty acids than did milk from full-term mothers. These fatty acids provide ready energy for growth.

Both Bitman and Wood are with the Milk Secretion and Mastitis Laboratory, Beltsville, Md.

"Our studies indicate that pre-term milk may be more adapted to the immature infant's digestive system," says Bitman. The large fat molecules in mature milk are more difficult to break down because the premature baby's digestive system has very low levels of bile salts and an enzyme—pancreatic lipase.

During the first 2 to 3 months of lactation, the pre-term child ingesting its own mother's milk receives about 17 percent of its total fat intake as fatty acids of medium-chain length whereas full-term infants receive about 10 percent.

Earlier studies have largely overlooked the benefits of premature milk, Bitman says, due to the limited amounts available for analysis. Samples were generally taken from full-term mothers or from human milk banks where milk from several donors is mixed.

Bitman and Wood also uncovered a storage problem when they froze samples for later analysis. It appears that lipases present in milk caused fat molecules to decompose even at conventional freezer temperatures. The best way to avoid breakdown of fat molecules in human milk is to freeze it to −70° C (−94° F) or lower, Bitman says.

Joel Bitman is located at the Milk Secretion and Mastitis Laboratory, Rm. 202, Bldg. 173, Beltsville Agricultural Research Center—East, Beltsville, Md. 20705.—**Lloyd McLaughlin,** Beltsville, Md. ■

AgResearch Notes

More on Trace Elements: Vanadium

Whether humans require any of the trace mineral vanadium has not yet been determined; but rats and chicks have often served as reliable models for studying human dietary needs, and they require an estimated 50 parts per billion of the mineral.

Recent research on rats at the Grand Forks Human Nutrition Research Center indicates that vanadium may influence several biochemical processes, including cholesterol concentrations in blood plasma and how copper is metabolized.

Without a thorough knowledge about relationships among dietary components, says biochemist Forrest H. Nielsen, attempts to supplement diets with trace minerals could sometimes be ineffective or even detrimental to health.

In the latest experiment, rats developed high concentrations of cholesterol in their blood when they ate diets high in vanadium and low in copper. Rats fed diets low in both these substances developed even higher blood plasma cholesterol concentrations. Rats on diets that had low but adequate levels of a particular amino acid, methionine, but high in vanadium and copper, developed low cholesterol concentrations. Amino acids are the chief components of proteins, the essential constitutents of living cells.

"Our findings indicate that dietary vanadium affects copper metabolism or perhaps dietary copper affects vanadium metabolism," Nielsen says. "However, the effects are markedly influenced by certain amino acids."

The study also showed that when rats deprived of adequate vanadium ate a low copper diet, they had increased iron concentrations in their livers. Because copper and iron metabolism interact, vanadium might affect iron metabolism through its effect on copper, Nielsen suggests.

If additional research shows that vanadium is essential in human diets, scientists may need to reassess diets for nutritional adequacy, Nielsen says. Foods low in vanadium include potatoes, butter, and ground beef. Foods rich in vanadium include black pepper, oysters, spinach, and peanut butter. Generally, food from plant material contains more vanadium than meats.

Other studies have shown that the vanadium content of foods is related to vanadium concentrations in soils where the foods are produced. For example, plant physiologist Ross M. Welch and Earle E. Cary of the U.S. Plant, Soil and Nutrition Laboratory, Ithaca, N.Y., found that wheat grown near Bushland and Denton, Tex., and in shale-derived soils near Quinn and Presho, S.Dak., contained considerably more vanadium than did wheat grown at eight other locations in Texas, South Dakota, Kansas, Minnesota, and Montana.

Forrest H. Nielsen is located at the Grand Forks Human Nutrition Research Center, P.O. Box 7166, University of North Dakota, University Station, Grand Forks, N.Dak. 58202.—**Ben Hardin**, Peoria, Ill. ■

Sniffing for Forbidden Fruits

Visitors to the 1984 Olympics who entered the country at the Los Angeles International Airport had their luggage electronically "sniffed" for contraband fruits and vegetables by a hand-held detector.

Designed to speed up baggage inspection, the suitcase-sniffing "gun" activates a warning light when it detects high levels of carbon dioxide, CO_2, in luggage. Plant materials give off CO_2.

The detector, developed by chemist Paul Magidman, engineer Wolfgang Heiland, and colleagues at ARS's Eastern Regional Research Center near Philadelphia, Pa., could help reduce the amount of undeclared plant material that slips by officials.

In a 2-day field trial prior to the extended trial during the Olympic games, Animal and Plant Health Inspection Service (APHIS) inspectors at the Los Angeles airport used the device to test about 90 pieces of luggage. Of these, 15 percent contained detectable agricultural material. The overall accuracy of the device was more than 90 percent. Because the unit was used in an area where most luggage is opened for visual inspection, inspectors could verify the accuracy of negative responses given by the detector.

ARS' prototype "sniffer" gun for detecting contraband fruit is tested at Los Angeles International Airport by USDA Animal and Plant Health Inspection Service officers Victor Johnson and Robert King. (0684X906-12)

The problem of insects and diseases foreign to U.S. agriculture hitchhiking into the country on fruits and vegetables in travelers' baggage is a constant one.

Prohibited fruit accounted for roughly two-thirds of the 476,000 plant products seized at U.S. international airports in 1983, according to APHIS officials. Over 31,000 insects were sifted out of the seized materials.

It is also possible that Mediterranean fruit flies may have come to the West Coast in 1980 in someone's luggage, says Gary Snyder, an APHIS staff officer. Eradication of the resulting Medfly infestation of fruit-growing areas cost federal and California governments approximately $100 million, he says.

Paul Magidman is located at the Eastern Regional Research Center, 600 E. Mermaid La., Philadelphia, Pa. 19118. —**Stephen Berberich,** Beltsville, Md.■

Breaking the Gossypol Barrier

Scientists have completed two important steps toward producing the ideal cotton plant—one with gossypol glands in the leaves and stems but not in the seed.

Gossypol is a toxic compound that remains in cottonseed oil and meal after processing. Removing the compound, which is poisonous to nonruminant animals such as pigs and chickens as well as to humans, is a troublesome and costly business. If a commercial cotton produced seed with no gossypol, the meal could be directly used as food or feed and the oil would require less processing.

Since the 1950's, scientists have had a cotton line that is completely free of gossypol. However, this "glandless" cotton (the toxic terpenoid chemicals associated with the yellow pigment gossypol are stored in small glands) lacked resistance to insect pests. Gossypol, it turns out, serves as an important protective mechanism against insects.

A breakthrough came in 1979 when geneticist Robert H. Dilday discovered a single plant growing in a field in Tucson, Ariz., that had gossypol glands in the foliage and flowers but none in the embryo. The plant was the result of crosses involving three cotton species having different numbers of chromosomes—a hexaploid, the commercial tetraploid *Gossypium hirsutum,* and a wild Australian diploid.

Starting with four seeds in 1979, Dilday persevered until he had a population of several thousand plants from which he has developed a fertile hybrid that has glands in the vegetative parts and is glandless in the seed. He hopes to complete the transfer into a commercial variety.

Says Dilday, "It took 40 years to transfer disease resistance into commercially acceptable cotton, so we know a laborious task lies ahead. However, what we are trying to do is definitive, not subjective, as was screening for disease resistance and fiber strength. That should make the job easier and quicker."

Robert H. Dilday is located at the Rice Research and Extension Center, P.O. Box 287, Stuttgart, Ark. 72160.—**Bennett Carriere,** New Orleans, La.■

Wild Mustard Seed Wins the Battle

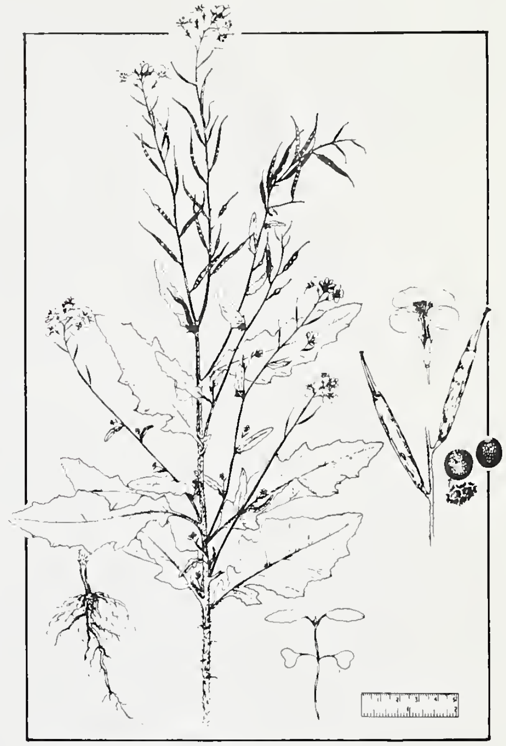
What is the best thing you can do to wear out wild mustard seed?

In an effort to find out, Dennis D. Warnes, agronomist with the University of Minnesota Agricultural Experiment Station, Morris, Minn., and Robert N. Andersen, ARS agronomist at St. Paul, worked out nine different cultural and chemical treatments and forced them on a wild mustard patch at Morris.

"Sad to say," Warnes says, "after 7 years of tests, the most effective treatment proved to be an impractical combination of three plowings and constant summer fallow treatments with a field cultivator. When we sampled the top 12 inches of soil, we found we had reduced the wild mustard seed by 97 percent. However, that remaining 3 percent worked out to 5.9 million seeds per acre."

Wild mustard is a serious problem in spring-seeded small grains in western Minnesota and in North and South Dakota, Warnes explains. Recent improvements in herbicides have provided control for wild mustard in soybeans.

"Unfortunately," Andersen says, "our results suggest that eradication of wild



Wild mustard *(Brassica kaber).* PN-7132

mustard from an infested field is impractical with currently available techniques."

Warnes says the objective was to determine the time required to eradicate wild mustard seeds from the soil. In undisturbed plots, about half of the seed population remains after seven growing seasons.

The scientists tested annual spring applications of 2,4-D or MCPA, plowing, chiseling, disking, field cultivation, and shallow cultivation in row crops. Crops included corn, soybeans, wheat, and continuous bromegrass.

In 3 of the 7 years, the researchers took soil samples to 18-inch depths and found wild mustard seeds below the plow layer. Andersen speculated that the seeds may have moved down through cracks in the soil during dry periods via channels left by decaying roots or by activities of soil organisms.

Although the number of seeds below the plow layer was insignificant when compared with that of seeds above, they could be important in restoring the wild mustard population if they were brought nearer the surface by deep plowing.

Robert N. Andersen is located at the ARS Plant Science Research Laboratory, University of Minnesota, St. Paul, Minn. 55108.—**Ray Pierce,** Peoria, Ill.■

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Patents

PATENTS is a regular feature of *Agricultural Research* magazine. Its purpose is to make the more than 1,200 patented inventions of the U.S. Department of Agriculture better known to businesses and individuals that might benefit from using them.

If you would like further technical information on a particular invention, contact the scientist whose name and address appear at the end of each item.

If you are interested in applying to obtain the license on a patent, write to the following address for an application form and information on license provisions and licensee responsibilities: Patents Office, USDA-ARS, Office of the Administator, Rm. 323, Bldg. 003, Beltsville Agricultural Research Center—West, Beltsville, Md. 20705.

Low-Cost Process for Heating Protein Solutions

The heating step used in separating proteins from liquid solutions can now be accomplished with low-cost heat exchangers.

The new process uses the exchangers to heat a portion of the solution that has already had the proteins removed. When mixed with the protein solution, the superheated liquid raises the overall temperature to a point where the proteins coagulate and precipitate out. It therefore eliminates the dilution problem associated with the use of steam to heat the solution.

The invention should be of particular interest to those who extract proteins from fresh forage, leafy green crops, and grains. Similarily, it can be useful in reducing pollution from food-processing waste water. In addition, it can be used in processes to recover proteins from animal liquids such as blood and egg albumen.

For further technical information, contact Richard H. Edwards, Western Regional Research Center, 800 Buchanan St., Berkeley, Calif. 94710. **Patent No. 4,421,682, "Heating of Proteinaceous Liquids."**

New Miticides Safer for Livestock

Manufacturers of agricultural chemicals should take note of the discovery of a series of compounds that control scabies mites and other parasitic mites as effectively as toxaphene, the most popular anti-scabies cattle dip, which is now being phased out by the Environmental Protection Agency.

The new compounds—all alkyl carbamates—were nontoxic to animals in preliminary toxicity tests. They can be made from very inexpensive, commercially available starter chemicals by several methods familiar to synthetic chemists.

Highly contagious scabies is an increasing threat to the U.S. cattle industry and a serious problem for sheep producers in several European and African nations. Only five chemicals, including toxaphene, are registered for its control in the United States, and most present disadvantages with their use.

For further technical information, contact Jan P. Kochansky, Rm. 106, Bldg. 467, Beltsville Agricultural Research Center—East, Beltsville, Md. 20705. **Patent Application Serial No. 443,995, "Control of Parasitic Mites with Alkyl Carbamates."**

Protection for Evergreens Against Bagworms

Discovery of the sex pheromone of the female bagworm moth can lead to nonpolluting products that protect landscape conifers—juniper, arborvitae, spruce, and white pine—from their most serious defoliator in the Eastern United States.

Because adult female moths remain within their bags throughout their lives, they rely on the sex pheromone to attract males during a short mating period in late summer. The synthetic pheromone is highly effective at low rates in trapping male moths; however, when used at high rates it prevents them from locating females via a special formulation that overloads their senses.

Companies that specialize in formulating pest control chemicals will find this pheromone is one of the simplest insect pheromones to make, costing only pennies per gram.

For further technical information, contact John W. Neal, Jr., Bldg. 470, Beltsville Agricultural Research Center—East, Beltsville, Md. 20705. **Patent Application Serial No. 445,112, "Bagworm Moth Attractant and Plant Protectant."**

Postharvest Biocontrol of Brown Rot

See "Giving Peaches a New Lease on Shelf Life," p. 12, for information on **Patent Application Serial No. 606,069, "Postharvest Biological Control of Stone Fruit Brown Rot by *Bacillus subtilis.*"**